Salmonella prevalence and antimicrobial resistance in swine from 5 US states from 2003 to 2005

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Abstract

The Collaboration in Animal Health and Food Safety Epidemiology (CAHFSE), a USDA joint program of ARS, APHIS, and FSIS was established to track food borne pathogens and monitor animal health issues. Fecal samples (n=9020) were collected and cultured for *Salmonella* from pens of pigs near slaughter weight (generally ≥ 22 weeks old) from swine farms in five U.S. states. A prevalence of 8.0, 10.1, and 8.5% was observed in 2003, 2004, and 2005, respectively. The top 10 serotypes accounted for 94% of the total *Salmonella* isolates with *S.* Derby (45%), *S.* Typhimurium var. 5- (15%), and *S.* Heidelberg (9%) comprising the top three serotypes each year. *Salmonella* Give was found in 8% of samples in 2003, 3% of samples in 2004, but was not found in the top 10% of isolates in 2005. The percentage of *Salmonella* isolates that were susceptible to all of the 16 antimicrobials tested increased from 6% in 2003 to 15% in 2005. At the same time, the percentage of isolates resistant to 10 or more antimicrobials increased from 1% to 15%. The increase in multiple drug resistance was coincident with an increase in the percentage of *S.* Derby isolates. Overall, frequency of resistance to individual antimicrobials was relatively stable from 2003 to 2005 and observed differences were related to changes in serotypes over time, which highlights the importance of reporting resistance data by individual serotype. CAHFSE provides a mechanism to monitor changes in serotypes of *Salmonella* as well as antimicrobial resistance patterns over time.

Introduction

Salmonella have been linked to food animal production and pork products are considered to be potential sources of *Salmonella* (White, et al. 2001). Nontyphoidal *Salmonella* spp. are estimated to account for 1.4 million cases of gastroenteritis in humans annually in the United States (Mead et al. 1999). Most cases result in self-limiting diarrheal disease. However, prolonged duration of illness, septicemia or altered immune function in some individuals may warrant use of antimicrobial therapy (Conte, 1995). Therefore, it is important to maintain an effective array of antimicrobials for potential treatment of bacterial infections.

The emergence of antimicrobial resistance in zoonotic bacteria associated with food producing animals, and evidence of human infections from animal sources (Fey, et al., 2000; Cohen and Tauxe, 1986) has spurred public health officials and scientists to reassess antimicrobial use in food animal production (FDA, 1998; WHO, 1997). In food animal production, antimicrobials are used both therapeutically and non-therapeutically. It is believed that therapeutic treatment of individual animals plays a minor role in the development of resistance. However, prolonged exposure of animals to non-therapeutic levels of antimicrobials for the prevention of disease and performance enhancement is believed to have the potential to increase antimicrobial resistance.

The results of the National Animal Health Monitoring System's (NAHMS) Swine 2000 study indicated antimicrobials were given in feed to grower/finisher pigs on 88.5% of the swine operations (APHIS, 2002) accounting for 95.9% of the grower/finisher pigs in the United States. Thus, antimicrobial use and related issues are a major concern to the pork industry. The merit and consequences of both therapeutic and non-therapeutic use of antimicrobials is under increasing scrutiny, but little information is available comparing the effects of these usage levels on the development and persistence of antimicrobial resistance among food borne pathogenic bacteria.

Previous monitoring programs have consisted of short-term studies of the presence of antimicrobial resistant populations, particularly in zoonotic pathogens associated with farm animals. To enhance and expand these initial monitoring efforts, a multi-agency "Public Health Action Plan to Combat Antimicrobial Resistance" was developed to address the potentially adverse effects of using antimicrobials in food animal production. The United States Department of Agriculture (USDA) responded by developing the Collaboration on Animal Health and Food Safety Epidemiology (CAHFSE), a partnership among USDA agencies; Animal and Plant Health Inspection Service (APHIS), Agricultural Research Service (ARS) and Food Safety and Inspection Service (FSIS). The primary objectives of CAHFSE are: 1) to enhance the overall understanding of pathogens that pose a food-safety risk by tracking these pathogens from the farm to the plant and 2) to monitor critical diseases in food-animal production. These objectives and critical issues related to the relationship between antimicrobial susceptibility and antimicrobial use will be addressed on a long term continuous basis under the CAHFSE program. Swine were the first commodity tested in the CAHFSE program.

Materials and Methods

On-Farm Sampling

CAHFSE sampling began in July, 2003 and by December 31, 2005, a total of 9020 fecal samples from 5 states (Iowa, Minnesota, Missouri, North Carolina and Texas) were tested for the presence of *Salmonella*. Selection criteria for soliciting farm participation included production types (indoor farrow-to-finish, outdoor farrow-to-finish, indoor finish only, and outdoor finish only) and size (number of pigs marketed per year; small $\leq$ 2,000, medium > 2,000 and $\leq$ 7,500, large > 7,500). Samples and data were collected quarterly. During each site visit, a questionnaire regarding animal inventory, animal health, management practices and antimicrobial use was completed.

Up to 40 pen floor fecal samples were collected from pigs at least 22 wks old for isolation and subsequent characterization of *Salmonella*. At least 5 samples per pen (center and at each corner) were taken for each of 8 pens. When there were less than 8 pens, then two or more sets of samples were taken from the same pen. Approximately 25 gm fecal samples were collected with a clean tongue depressor and placed in Whirl Pack bags. Liquid diarrhea fecal samples were placed in 50 ml centrifuge tubes, the screw caps were tightly secured and also placed in Whirl Pack bags. Samples were then shipped overnight on frozen cold packs to the Richard B. Russell Agriculture Research Center in Athens, Georgia.

Salmonella

Feces (1 g) was incubated in 10 mL of GN Hajna (Difco, Becton Dickenson, Sparks, MD) for 18-24 h at 37° C, and Tetrathionate broth (Difco) for 40-48 h at 37° C. After the initial enrichments, aliquots (100 µl) were transferred to 10 mL of Rappaport-Vassiliadis R10 broth (Difco) which were incubated for 18-24 h at 37° C. Ten microliter aliquots of Rappaport-Vassiliadis R10 broth were then streaked onto Xylose-Lysine-Tergitol-4 (Difco) and BG Sulfa (Difco) agar for isolation of *Salmonella*. Plates were incubated for 18-24 h at 37° C. Isolated colonies characteristic of *Salmonella* were inoculated into triple sugar iron and lysine iron agar slants for biochemical confirmation. Presumptive positive isolates were serogrouped using serogroup specific antisera (Difco) and were then sent to the National Veterinary Services Laboratory (Ames, IA) for serotyping.

Antimicrobial Susceptibility Testing

Salmonella, generic *E. coli* and *Enterococcus* antimicrobial susceptibility testing were conducted using the SensititreTM System (Trek Diagnostics, Inc., Westlake, Ohio) as per manufacturer's directions. Antimicrobials included those used in both human and veterinary medicine and were configured in a 96 well custom made panel. National Committee for Clinical Standards (NCCLS) (renamed to Clinical and Laboratory Standards Institute's (CLSI)) guidelines and resistance breakpoints were used throughout the testing procedure.

Results

Salmonella were recovered from 8.0, 10.1, and 8.5% of tested U.S. pigs in 2003, 2004, and 2005, respectively (Table 1).

Table 1: *Salmonella* prevalence 22 wk-old-pigs from five U.S. states

	2003	2004	2005
Samples tested	1,763	3,377	3,881
Number of positives	143	338	330
Prevalence	8.1%	10.0%	8.5%

The top 10 serotypes accounted for 94% of the total *Salmonella* isolates with *S.* Derby (45%), *S.* Typhimurium var. 5- (15%), and *S.* Heidelberg (9%) comprising the top three serotypes each year. *Salmonella* Give was found in 8% of samples in 2003, 3% of samples in 2004, but was not found in the top 10% of isolates in 2005 (Table 2).

Table 2: Predominate serotypes of *Salmonella* from U.S. pigs, 2003-2005

Rank	2003 n=146		2004 n=356		2005 n=346	
	Serotype	Percent	Serotype	Percent	Serotype	Percent
1	Derby	31.0%	Derby	45.21%	Derby	43.93%
2	Typh var 5-	27.6%	Typh var 5-	13.86%	Heidelberg	11.85%
3	Heidelberg	8.97%	Heidelberg	7.92%	Typh var 5-	11.56%
4	Give	7.59%	Typhimurium	6.60%	Typhimurium	7.51%
5	Mbandaka	6.90%	Untypable	4.29%	Mbandaka	6.65%
6	Typhimurium	4.83%	Give	3.96%	Agona	4.62%
7	Untypable	3.45%	Mbandaka	3.30%	Worthington	3.47%
8	Infantis	2.07%	Anatum	3.30%	Untypable	2.60%
9	Worthington	1.38%	Bovis-Morbificans	2.64%	4,5,12:i:-	2.02%
10	Bovis-Morbificans	1.38%	Worthington	2.31%	Newport	1.16%

The percentage of *Salmonella* isolates that were susceptible to all of the 16 antimicrobials tested increased from 6% in 2003 to 15% in 2005. At the same time, the percentage of isolates resistant to 10 or more antimicrobials increased from 1% to 15%. The increase in multiple drug resistance was coincident with an increase in the percentage of *S.* Derby isolates (Table 3).

Table 3: Multiple antimicrobial susceptibility of *Salmonella* from U.S. pigs, 2003-2005

No. of ABX	2003 n=146	2004 n=356	2005 n=346
Pan-Susceptible	5.5	5.3	15.0
1	29.7	33.3	10.7
> 2	64.8	61.4	74.3
> 5	21.4	28.7	30.6
>10	1.4	9.5	15.2

Discussion

Salmonella prevalence in swine fecal samples were similar to earlier reports (Bush et al., 1999). In addition *Salmonella* serotypes recovered in this study were typical of those reported in U.S. swine production. Resistance among *Salmonella* isolates was observed most frequently among antimicrobial agents used extensively in the past (streptomycin, sulfonamides, and tetracycline). Overall, frequency of resistance to individual antimicrobials was relatively stable from 2003 to 2005 and observed differences were related to changes in serotypes over time, which highlights the importance of reporting resistance data by individual serotype. Quinolones are not approved for use in swine in the U.S. and no isolates resistant to ciprofloxacin were observed. Since any use of antimicrobials can result in selection of resistant bacterial populations, antibiotics should only be used when warranted to treat disease or to enhance the healthy growth of animals.

CAHFSE provides a mechanism to monitor changes in serotypes of *Salmonella* as well as antimicrobial resistance patterns over time.

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